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PEROXIDASE ANTI-PEROXIDASE (PAP) STAINING
TECHNIQUE APPLIED TO THE VIRUS CLONING OF
HEMORRHAGIC FEVER WITH RENAL
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Kazuhiko YONEKURA

*Department of Veterinary Public Health
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan*

Most strains of hemorrhagic fever with renal syndrome (HFRS) viruses usually do not cause a definite cytopathic effect (CPE) in cell culture. Therefore, it is very difficult for these viruses to be cloned with plaque purification. In this study, a new virus cloning method was developed by the combined use of limiting dilution and peroxidase anti-peroxidase (PAP) staining technique (which is an enzyme-labeled antibody technique). Furthermore, the antigenicities and the infectivities of the cloned virus were compared with the original virus.

The results were summarized as follows ;

1. The stained foci could be detected clearly by the PAP method 5 days after infection in cultures with strain SR-11 and 7 days after infection in cultures with strain KI-262.
2. Eight clones were purified from strain SR-11 and their infectious virus titers were about 25 to 200 times higher than that of the original virus. Three of 8 clones produced CPE and plaques which could not be observed in culture inoculated with the original virus.
3. On the other hand, infectious titers of 11 clones from strain KI-262 were as low as that of the original virus and these clones did not produce CPE.
4. In indirect immunofluorescent antibody tests with monoclonal antibodies, the antigenicities of cloned viruses of both strains SR-11 and KI-262 differed slightly from original viruses. In neutralizing tests with rat immune serum, however, there was no difference in antigenicity between original viruses and cloned viruses.
5. When infectious titers of strains SR-11, KI-262 and H-76-118 were measured after trypsin treatment, strain H-76-118, isolated from *Apodemus*, was more resistant than SR-11 and KI-262 isolated from *Rattus*.
6. When strains SR-11, KI-262 and H-76-118 were absorbed to the cultured cells at low pH, the infectivity of strain H-76-118 differed from those of other strains.